



CLINICAL STUDY PROTOCOL

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Title: A Randomised Open Label Exploratory, Safety and Tolerability Study with PP100-01 in Patients Treated with the 12-hour Regimen of N-Acetylcysteine for Paracetamol/Acetaminophen Overdose

Short Title: **PP**100-01 (calmangafodipir) for **O**verdose of **P**aracetamol (The POP Trial)

Indication: Treatment of Paracetamol Overdose

Investigational product: PP100-01

Development phase: Phase 1

Coordinating Investigator: MD James Dear, University of Edinburgh, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland Tel: +44 0131 242 9214 Email: james.dear@ed.ac.uk

Project Manager: Dennis Henriksen, PledPharma AB

Sponsor: PledPharma AB, Grev Turegatan 11C, SE-114 46 Stockholm, Sweden

Contract Research Organisation: Edinburgh Clinical Trials Unit, Outpatients Building, Level 2, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU

ECTU Trial Manager: Kat Oatey Tel: +44 0131 537 3841, Email: k.oatey@ed.ac.uk

Research Nurse: Bernadette Gallagher: Emergency Department, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh EH16 4SA Email: Bernadette.Gallagher@nhslothian.scot.nhs.uk

Original Protocol Author: Dennis Henriksen, Email: dennis.henriksen@pledpharma.se and James Dear, Email: james.dear@ed.ac.uk

Statistician: Professor Christopher Weir, Tel: +44 01315373850, Email: christopher.weir@ed.ac.uk

Pharmacovigilance Manager (for SAE reporting): Nikkita Isichei, PRODUCTLIFE Ltd, The Jeffreys Building, St John's Innovation Park, Cowley Road, Cambridge, CB4 0DS, United Kingdom, Tel: +44 (0) 1223 402 660, Fax: +44 (0) 1223 413 689, Web: www.productlife-group.com, Email: safety@productlife-group.com

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with:

- The Guidelines of the World Medical Association (WMA) Declaration of Helsinki (as amended by the 59th WMA General Assembly, Seoul, October 2008)
- The Guidelines of Good Clinical Practice (GCP) (CPMP/ICH/135/95) Explanatory Note and Comments to the above, issued as CPMP/768/9
- EU Directive (2005/28/EG, April 2005)
- Demands of national drug and data protection laws and other applicable regulatory requirements

CONFIDENTIALITY STATEMENT

This Clinical Study Protocol (CSP) contains information that is confidential and proprietary to PledPharma AB (PPAB). This information is being provided to you for the purpose of conducting a clinical study for PPAB. You may disclose the contents of this CSP to study personnel under your supervision who need to know the contents for this purpose, as well as to your Independent Research Ethics Committee (REC). The contents of this CSP may not be disclosed to any other person or entity without the prior written permission of PPAB and may not be used for any other purpose than the conduct of this study. The foregoing shall not apply to disclosure required by governmental regulations or laws; however, you will give prompt notice to PPAB of any such disclosure.

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Any person who receives this CSP without due authorisation from PPAB is requested to return it to PPAB or to promptly destroy it.

SIGNATURES

INVESTIGATOR STATEMENT

I have read and understood this Protocol and agree to conduct the study accordingly. I have read and agree to comply with the Investigator obligations stated in this CSP.

I understand that deviations from the Protocol are to be noted and continuation must have the prior written approval by me, PledPharma AB (PPAB) and Edinburgh Clinical Trials Unit.

I agree to ensure that all personnel that assist me in the conduct of the study are aware of their obligations. I agree to use the study material, including medication, only as specified in the CSP.

I am thoroughly familiar with the appropriate use of the study drug, as described in this CSP and any other information provided by PPAB including, but not limited to, the current Investigator's Brochure (IB).

I agree to report any Serious Adverse Event (SAE) to the Sponsor as described in this CSP.

I am aware of, and will comply with GCP and all applicable regulatory requirements.

The signature below constitutes the approval of this CSP and appendices, and provides the necessary assurances that this study will be conducted accordingly.

Principal Investigator: Dr James Dear

Signature

Date

Printed Name

Site

PROTOCOL APPROVAL

An Open Label Exploratory, Safety and Tolerability Study with PP100-01 in Patients Treated with the 12-hour Regimen of N-Acetylcysteine for Paracetamol/Acetaminophen Overdose
EudraCT number: 2017-000246-21

Signatures

Dr James Dear
Chief Investigator/
Principal Investigator

Signature

Date

Dr Chris Weir
Trial Statistician

Signature

Date

Mr Dennis Henriksen
Sponsor(s) Representative

Signature

Date

STUDY PROTOCOL SYNOPSIS

Study Title: A Randomised Open Label Exploratory, Safety and Tolerability Study with PP100-01 in Patients Treated with the 12-hour Regimen of N-Acetylcysteine for Paracetamol/Acetaminophen Overdose

Protocol Number/Study Code: PP100-001

EudraCT Number: 2017-000246-21

Phase of Development: Phase 1

Name and Address of Sponsor: PledPharma AB, Grev Turegatan 11C, SE-114 46 Stockholm, Sweden

Coordinating Principal Investigator: James Dear, University of Edinburgh, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland.

Study treatment:

Each treatment of NAC or PP100-01 is administered intravenously. PP100-01 will be infused as a single dose over approximately 5 minutes.

N-Acetylcysteine (NAC) for paracetamol/acetaminophen overdose will be administered according to the 12-hr procedure with the addition of PP100-01;

1. Start "loading" dose: 100 mg/kg NAC diluted in 200 ml diluent* i.v. over 2 hours
2. PP100-01 bolus infusion over 5 minutes
3. Second dose: 200 mg/kg NAC diluted in 1000 ml diluent* i.v. over 10 hours

* diluent is 5% Dextrose or 0.9% Sodium Chloride.

Investigational Medicinal Product: PP100-01 given as one dose after the initial "loading" dose with NAC.

- Group A: PP100-01 (2 µmol/kg calmagafodipir) after the "loading" dose of NAC
- Group B: PP100-01 (5 µmol/kg calmagafodipir) after the "loading" dose of NAC
- Group C: PP100-01 (10 µmol/kg calmagafodipir) after the "loading" dose of NAC

In each dosing cohort there will be 8 patients randomly allocated to PP100-01 and NAC (N=6) or NAC alone (N=2).

Patient population:

Any patient requiring treatment with NAC (confirmed by blood tests) for acute paracetamol/acetaminophen overdose arriving to the hospital < 24hr after paracetamol/acetaminophen overdose (POD).

Study Purpose:

The study with PP100-01 in combination with NAC is designed to determine safety and tolerability of PP100-01 when co-administered with NAC as compared to the 12-hr NAC treatment regime for patients that come to the hospital after an overdose of paracetamol/acetaminophen.

As a secondary objective possible efficacy will be explored by using experimental biomarkers in serum/plasma, such as CK18 and microRNA MiR122, GLDH, mitochondrial DNA and others that might give efficacy signals for PP100-01. The diagnosis of paracetamol/acetaminophen overdose will be made by ALT and paracetamol/acetaminophen measurements.

This study includes several comparisons;

- Safety and tolerability of PP100-01 + NAC vs NAC alone
- Assess hepatotoxicity in patients after POD as assessed using ALT, INR and experimental biomarkers of liver damage

Primary Objective:

Investigate the safety and tolerability of PP100-01 add-on treatment to the 12hr NAC treatment regime in patients treated for paracetamol/acetaminophen overdose (POD) when NAC treatment is initiated before 24hours post POD.

Secondary Objective:

Determine if there is evidence of PP100-01 having efficacy with regard to treatment of paracetamol-induced liver injury by measurement of conventional clinical biomarkers and novel experimental biomarkers.

Study Design:

The study will be an open label, randomised, exploratory, rising dose design, NAC controlled, phase 1 safety and tolerability study in patients treated with NAC for paracetamol/acetaminophen overdose.

Entry into the study will depend on the patient's blood results confirming the need for NAC. A total of 24 patients will be assigned into one of 3 dosing cohorts of 8 patients (N=6 for PP100-01 and NAC; N=2 for NAC alone).

The study will primarily evaluate safety and tolerability for treatment with PP100-01 in combination with NAC as compared to NAC alone.

Baseline Assessments:

Blood tests, vital signs, physical examination and an ECG will be completed as part of the baseline assessments.

NAC will be given to all patients arriving to the hospital according to the standard guidelines when paracetamol/acetaminophen overdose is suspected. All patients that arrive to the hospital within 24 hr of POD and require NAC treatment will be asked if they will participate in the study once their blood results are available. The following investigations will be performed as part of clinical care on arrival to hospital or 4hrs after overdose (whichever is the longer time):

- Paracetamol level in serum/plasma
- Urea and electrolytes and creatinine
- Liver function tests (LFTs) ALT, Bilirubin and ALP
- Clotting screen: INR and Prothrombin time (PT)

Treatment Phase:

Patients that have signed the informed consent will be treated with NAC and randomised to NAC alone (N=2 per dosing cohort) or PP100-01 and NAC (N=6 per dosing cohort)

- Group A: PP100-01 (2 µmol/kg calmangafodipir) after the "loading" dose of NAC
- Group B: PP100-01 (5 µmol/kg calmangafodipir) after the "loading" dose of NAC
- Group C: PP100-01 (10 µmol/kg calmangafodipir) after the "loading" dose of NAC

PP100-01 treatment is administered intravenously over 5 minutes.

The NAC regime will be continued with the second dose: 200 mg/kg NAC in 1000 ml i.v. over 10hr.

The following procedures should be performed during the treatment phase:

- ALT bilirubin and alkaline phosphatase (ALP)
- Prothrombin time and INR
- Serum creatinine
- Biomarkers: CK18 and microRNA MiR122, GLDH, mitochondrial DNA and others

End of treatment:

At the end of the 12hr NAC regimen the decision to continue NAC is made by:

- the ALT has more than doubled since the admission measurement, OR
- the ALT is two times the upper limit of normal or more, OR
- the INR is greater than 1.3 (in the absence of another cause, e.g. warfarin)
- paracetamol/acetaminophen concentration greater than 20 mg/mL

Patients that do not require further NAC treatment will remain in the hospital for further 10hr and have a blood sample taken after 8hr. This blood sample will be after 20hr of starting NAC.

Follow-up:

There will be no follow-up visit, but patients will be followed up using their electronic records. The following data are collected 7, 30 and 90 days after randomisation: Representation to hospital (any reason), representation with liver injury, repeat overdose, death and transfer to liver transplantation unit. Any AEs or SAES identified will be recorded and reported.

Inclusion Criteria:

1. Any patient with capacity admitted to hospital within 24 hrs either a single acute POD or more than one dose of paracetamol (staggered) and deemed to require treatment with NAC.
2. Provision of written informed consent
3. Males and females of at least 16 years of age

These patients will include:

- a) Patients presenting within 8h of overdose who have a timed blood paracetamol concentration above the 100-line on the UK APAP overdose treatment nomogram (See Figure 1).
- b) Patients presenting later than 8h who are at risk of liver injury from the reported dose ingested and have blood results confirming need for NAC
- c) Patients presenting after taking a staggered APAP overdose (defined as when the overdose of APAP is taken over a period of more than 1hr)

Exclusion Criteria:

1. Patients that do not have the capacity to consent to participate in the study
2. Patients detained under the Mental Health Act or deemed unfit by the Investigator to participate due to mental health.
3. Patients with known permanent cognitive impairment
4. Patients who are pregnant or nursing
5. Patients who have previously participated in the study
6. Unreliable history of POD
7. Patients presenting after 24hrs of POD
8. Patients who take anticoagulants (e.g. warfarin) therapeutically or have taken an overdose of anticoagulants
9. Patients who, in the opinion of the responsible clinician/nurse, are unlikely to complete the full course of NAC e.g. expressing wish to self-discharge
10. Prisoners
11. Non-English speaking patients. (Study information material will only be produced in English in view of the known and stable demographic of the Edinburgh self-harm population).

Study Procedures:

Independent Safety Data Monitoring Committee (SDMC):

An independent monitoring board will review safety data after each dosing cohort of PP100-01 has been completed and before the next dosing cohort can be initiated. The SDMC will assess safety, unexpected toxicities and unexpected increased severity of toxicities in the experimental arms. They will also propose changes to the protocol and/or stop the study if indicated. All further patient enrolment will be paused pending advise from the SDMC if one of the following stopping rules have been met: 1. Patient death, admission to a Critical Care Unit or admission to a Liver Transplantation Unit due to any reason, or 2. One SUSAR that definitely or probably relates to either PP100-01 or NAC or both.

Statistical Methods:

A CONSORT diagram depicting the flow of participants through the study will be reported. Descriptive statistics will be used to report baseline characteristics by treatment group (PP100-01 groups A, B, C, or NAC alone) and overall: continuous variables will be summarised by the mean, standard deviation, median minimum and maximum; categorical variables will be summarised using the number and percentage in each category. We will keep missing data to an absolute minimum, but where there is missing data those records will be removed from the analysis; if missing data rates are substantial the effect of this will be investigated using sensitivity analyses. Binary outcomes (including the primary outcome) will be reported by treatment group and overall using the proportion and exact 95% confidence interval. Continuous, outcomes will be reported by treatment group and overall using the mean and 95% confidence interval. The full details will be provided in a separate Statistical Analysis Plan.

Study schedule:

Visit windows	Screen ing	0 (NAC treatment)	Baseline (Pre-PP100-01)	After 2 hrs	After 2.5 hrs	After 10 hrs	After 12 hrs	After 20 hrs	Discharge after 22 hrs	7 day follow up	30 day follow up	90 day follow up
Informed Consent	X											
Eligibility Criteria	X											
Enroled into trial	X											
Treatment Allocation/randomisation	X											
Bloods												
APAP conc level	X					X		X				
INR /Prothrombin	X			X		X		X				
ALT (IU/L)	X			X		X		X				
ALP (IU/L)	X			X		X		X				
Bilirubin	X			X		X		X				
Creatinine	X			X		X		X				
Hb	X					X		X				
Urea	X					X		X				
Sodium	X					X		X				
Potassium	X					X		X				
MCV	X					X		X				
WBC	X					X		X				
Inflammation Biomarkers				X		X		X				
PP100-01 levels				X		X		X				
Pregnancy test	X											
Treatment												
NAC Treatment		X			X		(X)	(X)				
PP100-01 Treatment				X								
Physical Examination			X	X*	X*	X*		X*				
ECG			X		X	X		X				
Vital signs (BP and P)			X		X	X		X				
Adverse events			X	X	X	X		X	X	X	X	X

*If AEs/SAEs identified that require a full physical exam to be completed

(x) NAC continued as per TOXBASE (12hours +/-30mins)

2, 2.5hour assessments should be performed +/-15mins of the timepoint. PP100-01 treatment should be +/- 10mins of timepoint. 10, 20hour assessments should be performed +/-30minutes of the timepoint.

LIST OF ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
APAP	Paracetamol/Acetaminophen
AR	Adverse Reaction
AST	Aspartate amino transferase
BNF	British National Formulary
BP+P	Blood pressure and pulse
C _{max}	Maximum observed plasma drug concentration
CNS	Central nervous system
CRA	Clinical Research Associate
CRF	Case Report Form
CSP	Clinical Study Protocol
CSR	Clinical Study Report
DLT	Dose Limiting Toxicity
DM	Data Manager
ECG	Electrocardiography
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMA	The European Medicines Agency
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FDA	Food and Drugs Administration
FBC	Full Blood Count
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GSH	Glutathione
Hb	Haemoglobin
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
INR	International normalised ratio
ISF	Investigator Site File
ITT	Intention-To-Treat
IV	Intravenous
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LFTs	Liver Function tests
MCV	Mean cell volume
Mn	Manganese
MnSOD	Manganese superoxide dismutase
N&V	Nausea and vomiting
NAC	N-Acetylcysteine
NAPQI	N-acetyl-p-benzoquinoneimine
NPIS	National Poisons Information Service
NPIS(E)	National Poisons Information Service (Edinburgh)
OTC	Over the counter
PI	Principal Investigator
PIS	Patient Information Sheet
POD	Paracetamol/acetaminophen overdose
PONV	Post-operative nausea and vomiting
PP100-01	IMP containing calmangafodipir
q.s.	quantum satis
REC	Research Ethics Committee
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDMC	Safety data monitoring committee
SDV	Source Data Verification
SOD	Superoxide dismutase
SOP	Standard Operating Procedure
SoPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
T _{max}	Time of maximum observed plasma drug concentration
TMF	Trial Master File
U&E	Urea and electrolytes
UAR	Unexpected Adverse Reaction
ULN	Upper Limit of Normal
WBC	White Blood cell Count

CONFIDENTIAL

1 SUMMARY

1.1 Professional Summary

Paracetamol/acetaminophen (APAP) is a widely found intoxication in the United Kingdom and is present in approximately 40% of patients admitted with self-harm. Current treatment involves use of the antidote N-acetylcysteine (NAC) in patients deemed at risk of potential liver damage. This is given by intravenous infusion over a period of 20.25hrs. This regimen was designed in the 1970s and is empirical, in that a large loading dose of the antidote is administered followed by 2 decreasing concentrations. The initial infusion is associated with adverse reactions, in particular nausea and vomiting and anaphylactoid reactions. The latter are particularly troublesome and occur in up to 15% of patients treated. Therapy is discontinued and there is often confusion as to whether therapy can be restarted in a timely manner.

Trialling antidotes in the management of poisoning is challenging not least because of the patient population and of the limited time available to make decisions and gain consent.

The objective of this study is to develop a therapeutic regimen for paracetamol/acetaminophen overdose (POD) where a novel NAC 12hr regime is combined with a superoxide dismutase (SOD) mimetic, PP100-01 in order to evaluate if reduction of the oxidative stress on the liver will be safe and well tolerated. It will also allow preliminary data to be collected on a new approach of giving acetylcysteine together with PP100-01 using the 12hr regimen, which includes a slower initial intravenous infusion over 2hrs instead of 15 min in the 20.25hr NAC regime.

The primary study outcome will therefore inform on the safety and tolerability of PP100-01 co-treatment with NAC regime in patients with POD. In addition valuable data on the incidence of adverse effects caused by the modified NAC and PP100-01 regimen, and changes in liver function and the inflammatory response to APAP induced liver injury within this modified NAC treatment will be obtained. Blood samples will be taken from all patients to assess the exposure of NAC and PP100-01 as well as biomarkers of APAP toxicity and the inflammatory response.

In total a maximum of 24 patients will be recruited.

The demographic of this patient group is essentially Caucasian English-speaking and at this stage we do not propose to recruit non-English-speaking patients.

Lay Summary

Paracetamol/acetaminophen (APAP) is the most common agent taken in overdose by patients in the United Kingdom and hospitalisation due to POD accounted for approximately 80,000 bed days in the UK in 2005-2006 (Hospital Episode Statistics & ISD Scotland)

It is the most often seen cause of acute liver failure in the western world, but its toxicity is potentially preventable by timely intervention with the antidote N-acetylcysteine (NAC). This antidote was introduced in the early 1970s using an intravenous regimen with 3 doses given by infusion over 20.25hrs. Side effects include in particular nausea and vomiting in about 2/3 of patients, and anaphylactoid reactions. The latter consists of flushing, tachycardia, wheeze, and in more severe cases falls in blood pressure and chest pain.

In order to study this in more detail this project will evaluate a 12hr NAC treatment regimen and a new treatment protocol, which administers NAC together with a superoxide dismutase (SOD) mimetic, PP100-01 (calmangafodipir), where PP100-01 is given after the initial "loading" dose of NAC. The total NAC dose is the same as the standard NAC regime.

The protocol to be studied will include three separate treatment arms. These are:

- a) NAC regimen with PP100-01 (2 µmol/kg calmangafodipir) co-treatment
- b) NAC regimen with PP100-01 (5 µmol/kg calmangafodipir) co-treatment
- c) NAC regimen with PP100-01 (10 µmol/kg calmangafodipir) co-treatment

6 patients in each of three PP100-01 co-treatment with NAC arms (along with 2 NAC alone treatments) will be studied to explore safety and tolerability.

The patient will be given enough time to consider the study and ask questions regarding their participation in the study. Patients will have the potential to withdraw from the study at any time should they wish.

All patients will receive 12hr of intravenous therapy, and blood samples will be taken at this time, as is currently routine practice at the Royal Infirmary of Edinburgh when caring for patients with POD. Blood samples for safety monitoring. Three blood samples for safety monitoring will be obtained in all patients; one before administering PP100-01, one during the second NAC dose at the 10hr time-point after starting NAC treatment and the last at 20hrs after starting NAC treatment, which will be 2hrs prior to end of the study. The total study therapy length will be 22hrs.

This study may not benefit individual patients on this occasion, but will determine if PP100-01 co-treatment with NAC is tolerated.

Results from this study will also be used to design further studies with the PP100-01 co-treatment regimen with NAC.

Furthermore, the study is designed to provide evidence on biomarkers of APAP toxicity, including the body's inflammatory response to APAP-induced liver injury, together with data on the plasma concentrations of NAC and PP100-01 resulting from the treatment regimens used. Markers of APAP toxicity and inflammatory response will be assessed in all patients.

The demographic of this patient group is essentially Caucasian English-speaking and at this stage we do not propose to recruit non-English-speaking patients.

2 BACKGROUND INFORMATION

2.1 Paracetamol/acetaminophen Overdose

Paracetamol/acetaminophen (APAP) is a widely found drug taken in overdose in the United Kingdom, including in Scotland. Hospitalisation due to paracetamol/acetaminophen overdose (POD) accounted for approximately 80,000 bed days in the UK in 2005-2006 (Hospital Episode Statistics & ISD Scotland).

The management of patients who present with symptoms of POD early (less than 8hrs) after ingestion, is well established and guided by the plasma APAP concentration. Treatment with N-acetylcysteine (NAC) in patients with a toxic plasma APAP concentration provides optimal protection against APAP induced hepatotoxicity if treatment with NAC is initiated less than 8 hours post POD.

Patients that ingest an acute POD and have NAC treatment initiated within 8hrs post POD do well and have less than a 5-8% incidence of hepatotoxicity and generally do not develop liver failure or die. Those patients that chronically ingest excessive doses of APAP over many hours and/or have NAC therapy initiated more than 8 hrs after an acute overdose have an approximately 25% incidence of hepatotoxicity (Eriksson et al 1992, Green et al 2013, Senararhna 2012 and Brok et al 2013). Unlike when NAC therapy is initiated early, patients that have NAC administration after 8 hrs from the POD are at risk of developing fulminant hepatic failure and death.

In POD, the normal detoxification pathway (sulphation and glucuronidation) is overwhelmed, leading to the formation of the reactive intermediate metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), which binds covalently to liver proteins resulting in cell death. Thus, POD can cause hepatocellular injury leading to fulminant hepatic failure and death if untreated. In a cohort of 57 untreated patients hospitalised with POD, 33 (58%) developed severe hepatocellular injury and 3 (5%) died (Prescott et al 1979).

Most of the mechanistic insight into the pathophysiology of POD has been gained from experiments with the murine system, which closely resembles the human pathophysiology in terms of liver injury and recovery. In addition, the severity of the overall liver injury is very similar between mice and humans (Jaeschke et al 2014).

These experimental animal data show that APAP-induced toxicity occurs by two phases, an initial metabolic phase followed by an oxidative phase. In human patients the metabolic phase is predominantly during the first 8hrs (0-8 hrs) and the oxidative phase will dominate thereafter (>8hrs). During the metabolic phase, APAP metabolites are conjugated and excreted via the kidneys. In the oxidative phase the glutathione (GSH) stores have been depleted and the reactive metabolite NAPQI binds to liver proteins with increased oxidative stress and ultimately loss of mitochondrial membrane potential and subsequent cell death.

Furthermore, covalent binding of NAPQI to mitochondrial proteins is causing mitochondrial dysfunction that leads to inhibition of the mitochondrial respiration with enhanced formation of reactive oxygen species and peroxynitrite in mitochondria. The oxidant stress can directly trigger the mitochondrial membrane permeability with pore opening and collapse of the mitochondrial membrane potential. The toxicity of reactive oxygen and reactive nitrogen species is potentiated by the fact that mitochondrial GSH levels are severely depleted during the APAP metabolism, which leaves these cell organelles highly vulnerable (Singer et al 1995).

2.2 Acetylcysteine

Acetylcysteine (N-acetylcysteine; NAC) was developed as an antidote for APAP poisoning in Edinburgh in the 1970s and remains the mainstay for the prevention of APAP-induced hepatotoxicity. It is metabolised in the liver to the glutathione substrate cysteine. Glutathione is required for the detoxification of the toxic metabolite of APAP, NAPQI, and produce less toxic cysteine and mercapturic acid conjugates.

POD patients in the UK still receive the intravenous (IV) 20.25 hr regimen first used in Edinburgh by Prescott and colleagues in 1977: 150 mg/kg over 15 minutes, then 50 mg/kg over 4 hrs, then 100 mg/kg over 16 hrs (total dose 300 mg/kg) (Prescott et al 1977). This regimen was empirical and not based on any initial dose-ranging study.

Until 2004, when the IV regimen was approved by the FDA, only oral NAC was licensed in the United States and an oral regimen of NAC consisting of a loading dose of 140 mg/kg followed by 17 further doses of 70 mg/kg at 4 hourly intervals (total dose 1330 mg/kg, duration of treatment

72hrs) was widely used. Intravenous NAC is now used increasingly in the US in view of the shorter duration of treatment and less cumbersome treatment regime.

2.2.1 Efficacy of Acetylcysteine in Clinical Use

Before antidotal therapy became available in the 1970's, 58% of hospitalised patients treated supportively following POD developed hepatotoxicity and the mortality rate from fulminant failure was 6% (Brok et al 2006).

The initial studies with NAC showed that the 20.25h intravenous regimen was almost 100% effective in preventing hepatotoxicity when given within 8hrs of overdose and significantly more effective than no treatment as determined using historical controls (Prescott et al 1979). A large retrospective Canadian study of 1270 patients with POD treated mostly with this regimen showed that no deaths occurred, 1 patient received a liver transplant, 94 (7.4%) developed an ALT>1000 and 233 (18.3%) developed an ALT>100 post-treatment (Sivilotti et al 2005).

The use of NAC has reduced the overall rate of severe hepatotoxicity (conventionally defined as ALT>1000) to less than 20% and mortality to less than 1% (Brok et al 2006).

An essential factor for the efficacy of NAC treatment is the timing of therapy initiation in relation to the ingestion of APAP. The mechanism of APAP-induced toxicity, as already mentioned, is complex involving several phases including metabolic activation, covalent binding as well as an oxidative phase most likely involving non-parenchymal cells, (Reid et al., 2005). The metabolic phase includes metabolic activation, glutathione depletion and binding of the reactive APAP metabolite NAPQI to liver proteins. The oxidative phase results in increased oxidative stress, ultimately loss of mitochondrial membrane potential and subsequent cell death (Kon et al. 2004; Masubuchi et al., 2005; Reid et al., 2005). NAC provides protection in the early metabolic phase by supplying cysteine for the glutathione synthesis, which traps the reactive APAP metabolite, but NAC has reduced effect in the later oxidative phase.

2.2.2 Adverse Events in NAC Treatment

Although very effective for preventing APAP-induced hepatotoxicity, particularly when used within 8hrs of overdose, intravenous NAC is associated with adverse reactions and administration errors.

Depending on study type, selection criteria, definition of reactions, the rate of adverse reactions varies from <10% to over 50% (Lynch et al 2004; Mullins et al 2004), with adverse reactions commonly associated with the very high blood NAC concentration attained during the 150 mg/kg loading dose.

Nausea and vomiting are extremely common in patients receiving NAC, occurring in 70.4% and 60.4% respectively in a recent cohort of 169 patients treated in Edinburgh (Pakravan et al 2008). The mechanism underlying this common adverse reaction remains unclear, but it occurs in patients that develop hepatotoxicity and in those who do not.

In a randomised controlled study, the incidence of anaphylactoid adverse drug reactions was 18% and 14% in patients receiving the initial loading dose of 150 mg/kg NAC over 15 minutes and 60 minutes respectively (Kerr et al 2005). While this study lacked the statistical power necessary to exclude clinically important benefits from the slower initial infusion rate, the results suggest that the slower initial infusion rate should be considered. In the United States, the FDA advised in 2006 that the initial loading dose of NAC be given over 60 minutes.

Anaphylactoid reactions, characterised by symptoms such as rash, wheeze, and hypotension are thought to be dose and rate-related. Studies in Edinburgh have demonstrated that these are mediated by histamine release (Pakravan et al 2008). In one prospective randomised controlled study, the rate of anaphylactoid reactions was 18% (Kerr et al 2005). A recent prospective study of patients treated with the standard intravenous NAC regimen in the Edinburgh toxicology unit showed that around 15% developed anaphylactoid reactions (Pakravan 2008) and similar figures have been reported from Newcastle (Fathalla et al 2008). Anaphylactoid reactions are managed by interrupting the infusion temporarily and administering an antihistamine and bronchodilators. Most affected patients can be restarted on NAC at a lower infusion rate. Females, asthmatics and patients with low plasma APAP concentrations are at higher risk of anaphylactoid reactions (Schmidt et al 2001, Waring et al 2006, Pakravan 2008) and fatal adverse reactions have been reported, although these are rare.

Although highly effective, especially if started within a few hours of paracetamol overdose, the current licensed intravenous 20.25hr NAC regimen has several disadvantages. Firstly, intravenous NAC is commonly associated with adverse effects, the most common being nausea, vomiting and anaphylactoid reactions. Anaphylactoid reactions appear related to the rate of infusion of NAC and its concentration in blood. Thus they are most common during, or soon after, the initial high dose infusion. Secondly, the infusion schedule is complex, requiring prescription of three different infusions, and this contributes to a high risk of medication errors, which may have serious, potentially life-threatening, adverse outcomes. Thirdly, the required duration of the infusion regimen results in prolonged hospital stay, especially as patients with paracetamol overdose often present during the night (Thanacoody et al 2013).

To address these shortcomings of the standard 20.25hr NAC regime a shorten 12hr intravenous regimen of acetylcysteine has been developed. In this 12hr NAC regime the initial loading dose (NAC 100 mg/kg in 200 ml) is given over 2hrs, followed by a second dose (200 mg/kg in 1000 mL) infused over 10 hrs. This 12hr NAC regime has been shown to be effective at reducing the incidence of vomiting and anaphylactoid reactions, compared with the standard 20.25 h intravenous acetylcysteine schedule (Bateman et al 2014). The shorter duration of acetylcysteine infusion offers simpler administration, a probable reduction in administration errors, and a potential decrease in the length of the hospital stay.

2.3 PP100-01 (Calmangafodipir)

PP100-01, with the active pharmaceutical ingredient calmangafodipir ($\text{Ca}_4\text{Mn}(\text{DPDP})_5$), is a unique chemical species derived from *mangafodipir*, where 80% of the manganese in *mangafodipir* has been replaced with more readily displaceable calcium to form a mixed-metal complex to increase the proportion of fodipir-bound manganese following intravenous administration. It is important to note that calmangafodipir is a single API and is not a mixture of *mangafodipir* and calcium *fodipir*. *Mangafodipir* was originally developed as a contrast medium that has been approved by the FDA and EMA for diagnostic Magnetic Resonance Imaging (MRI) for the detection of lesions of the liver. When administered *in vivo*, readily available zinc in the bloodstream displaces approximately 80% of the manganese in *mangafodipir*, some of which was found to be deposited in the brain.

Mangafodipir has MnSOD (superoxide dismutase) activity which protects healthy cells from oxidative damage, and has been shown to have myeloprotective effects. In addition, mangafodipir protects against oxaliplatin-induced neurotoxicity and possesses cytotoxic activity against tumor cells. Although the contrast property of mangafodipir depends on the *in vivo* dissociation of Mn^{2+} from fodipir, the SOD mimetic activity of mangafodipir requires the intact Mn-fodipir complex (Brurok 1999; Karlsson 2001). Based on the similarities between calmangafodipir and mangafodipir, it is anticipated that calmangafodipir would exhibit SOD-dependent pharmacologic actions similar to those of mangafodipir.

Manganese superoxide dismutase (MnSOD) is located predominantly in the mitochondrial matrix and plays an important role in the detoxification of mitochondrial superoxide metabolites. Preventing the mitochondrial pore formation by scavenging reactive oxygen and peroxynitrite thereby reduced DNA damage (Fridovich 1998).

It has been shown that MnSOD mimetics/transition metal chelators offer protection in mice at a time point when NAC no longer gives protection, presumably corresponding with the oxidative phase (Bedda et al 2003). This in turn suggests that a MnSOD mimetic/transition metal chelators may provide protection against POD also in humans at a time when NAC treatment is less effective after 8-15hrs post POD.

Quality

The Active Pharmaceutical Ingredient (API) calmangafodipir is manufactured according to cGMP in a three step manufacturing process where the final step is single step crystallization of calcium and manganese with fodipir. PP100-01, the Investigational Medical product (IMP) is composed of the API in an aseptically prepared, sterile filtered, aqueous formulation for parenteral administration and produced according to cGMP.

PP100-01 must be stored at -15 to -25° C and protected from light. An ongoing stability program has to-date demonstrated that IMP is stable for at least 36 months at -20° C and 1 week +2 to 8° C. In addition, freeze /thaw investigations have been performed and the product

was shown to be stable (one additional freeze/thaw cycle and subsequent storage at 5°C±3°C for one week).

Non-clinical development

Since calmangafodipir is a "stabilized" form of mangafodipir with the same active ingredient but where 4/5 of the Mn is replaced with Ca and since identical circulating metabolites are formed after administration of the two compounds, an abridged non-clinical safety program has been conducted with calmangafodipir. This consisted of 13-week repeat dose toxicity studies in rats and dogs that included assessment of safety pharmacology endpoints. In order to support the use of the non-clinical safety information on mangafodipir, this compound was included as a reference compound in both the rat and dog 13-weeks toxicity studies.

No indication of any central nervous system or respiratory system effects was noted in rats after dosing with up to 37-fold the highest proposed clinical dose of 10 µmol/kg calmangafodipir. There were no indications of adverse effects on cardiovascular function (blood pressure and ECG) in dogs treated with i.v. doses of up to 31-fold the highest recommended clinical dose.

The repeat dose studies in rats and dogs included three dose levels of calmangafodipir and one with mangafodipir (dose equivalent to the high dose of calmangafodipir in regards to Mn). Animals were dosed 3 times a week; 39 administrations in total. On a Mn dose basis, rats given the high calmangafodipir dose were administered 37-fold the highest proposed clinical dose. High dose dogs were administered 31-fold the clinical dose. There were no changes in clinical pathology parameters that were considered drug-related or toxicologically significant in rats administered calmangafodipir. The absolute and/or relative weights of testes and epididymides were slightly lower in high dose animals. However, there were no clear pathological findings in these organs and their organ weights were similar to controls at the end of the recovery period. There were no calmangafodipir-related microscopic findings. Dogs administered calmangafodipir showed a dose and time-related loss of fur as well as hypersalivation and/or vomiting during the infusion with the high calmangafodipir dose. Body weight and body weight gain mean values were lower than controls in high dose males and females but showed evidence of recovery during the treatment free period. Histological examination showed testicular tubular atrophy in one high dose animal.

Fertility studies in male rats and embryofetal development studies in rats and rabbits have been conducted with mangafodipir. Skeletal malformations were observed in rats at the lowest dose investigated and embryofetal toxicity was observed in rabbits. Identical effects were seen after administration of equimolar MnCl₂. The genotoxicity was assessed using an ICH standard battery of tests as well as a cell transformation assay. Mangafodipir was determined to be non-genotoxic. It is the Sponsor's opinion that mangafodipir and calmangafodipir should have a similar genotoxic and reproduction toxicity liability.

Results from pharmacology studies show that calmangafodipir protects BALB/c mice against the myelosuppressive effects of oxaliplatin and that the analogue mangafodipir effectively prevents the onset of locomotor and sensory disturbances as well as neuromuscular hyperexcitability in C57Bl/6 mice treated with oxaliplatin.

Clinical development

Calmangafodipir as the API, has been studied in a Phase 2 safety and efficacy study (PLIANT) of CIPN in patients with advanced metastatic colorectal cancer. In the PLIANT study calmangafodipir (under the name, PledOx[®]) corresponding to 2, 5 or 10 µmol/kg was infused intravenously as a pre-treatment single dose over approximately 5 minutes. PledOx[®] was well tolerated across all three doses 2, 5 and 10 µmol/kg. PledOx[®] demonstrated an excellent safety profile with no meaningful differences between PledOx[®] treated arms and placebo in terms of adverse events. PledOx[®], in treatment dose of 5 µmol/kg decreased the incidence of physician-reported peripheral neuropathy (Sanofi-NCI oxaliplatin specific scale) of grade 2 or greater by approximately 40%. Additional analyses were undertaken and demonstrated that PledOx[®] delayed the onset and duration of Grade 2 or higher neuropathies compared with placebo. In addition, a statistically significant persistent treatment effect of 5 µmol/kg PledOx[®] was seen at the 12 and 24 week follow-up period from the patient reported assessment of neuropathy using the Leonard questionnaire (Leonard, 2005). This further distinguished the effect of PledOx[®] vs. placebo. These results were supported by an additional patient reported outcome, the Cold Allodynia Test (Ventzel, 2015). The consistency of the response between the physician reported neuropathy and the patient reported outcomes increase the robustness of the conclusion that PledOx[®] demonstrated a meaningful benefit in preventing the establishment of CIPN in this patient population. Additionally, PledOx[®] had no negative impact on cancer treatment response as indicated by the objective

response rates (RECIST 1.1) at the end of treatment. Finally, PledOx[®] demonstrated modest positive effects on the incidence of chemotherapy-induced cytopenias.

Calmangafodipir (PledOx[®]) had a safety profile in this study as would be predicted by non-clinical data. The most common preferred terms for safety reporting in the study (more than 25 events reported in total) were: neurotoxicity, thrombocytopenia, fatigue, nausea, neutropenia, peripheral sensory neuropathy, diarrhea, leukopenia, decreased appetite, anemia, paresthesia, neuropathy peripheral, vomiting and pyrexia. As expected, the frequencies of neurotoxicity, thrombocytopenia and neutropenia that were reported as AEs mirrored the reporting of these symptoms as chemotherapy-induced toxicities analyzed as efficacy data. Grade 2 neurotoxicity occurred in a smaller proportion of patients in the 5 µmol/kg PledOx[®] group (4.4%, 2 events) compared to placebo (16.7%, 16 events). No other apparent differences between the treatment groups in terms of adverse events.

To date, approximately 150 patients with mCRC received repetitive dosing with calmangafodipir (PledOx[®]). Approximately 240,000 patients have received single doses of the closely related compound mangafodipir at similar or higher doses. No new significant safety data have emerged during the treatment reporting period. Immediate blood pressure fall, severe diarrhoea and severe vomiting, have previously been identified as important potential risks, to be closely monitored as the clinical program progresses.

3 RATIONALE FOR THE STUDY

The objective of this study is to develop a therapeutic regimen for POD where NAC is combined with the SOD mimetic, PP100-01 (drug substance, calmangafodipir) in order to evaluate if reduction of the oxidative stress on the liver will be safe and well tolerated. Furthermore, the study will evaluate the dose response for PP100-01 using new experimental biomarkers in serum/plasma such as CK18 and microRNA (MiR122), GLDH and mitochondrial DNA to find new markers that identify organ injury earlier in the disease process than current markers and might give insight to the PP100-01 mechanism of action in POD.

The primary mode of action of PP100 is the inhibition of the formation of reactive oxygen species formed during oxidative phase after POD.

Standard therapy is currently NAC by intravenous infusion over 20.25hrs. This regimen is given to those deemed "at risk" using standard criteria (British National Formulary 2009). It has 4 major problems: 1) adverse events (nausea and vomiting and anaphylactoid reactions), 2) therapy duration, 3) complexity of administration increases the risk of reconstitution and administration errors and 4) diminished liver protection in late presenters (≥8hrs) as well as staggered POD.

Using the 12hr NAC protocol will simplify dose calculation involved as only two NAC doses will be needed and this may help reduce risks of over or under dosing. The 12hr NAC regimen administers the same total dose as the standard 20.25hr regime, but in 2 separate infusions instead of 3 and, therefore, may reduce the risks of error associated with preparing infusions.

An explorative aspect of the study will be to explore new experimental biomarkers in serum/plasma such as CK18 and microRNA (MiR122), GLDH and mitochondrial DNA to find new markers that identify organ injury earlier in the disease process than current markers.

The study will provide experience and data from a 12hr IV NAC regimen to design a study of the modified combined regimen as a new treatment for this common drug overdose. Such an approach has a major potential to reduce patient adverse events from NAC therapy and potentially lower the incidences of hepatotoxicity.

3.1 Routine Medical Management of POD

A nomogram (Figure 1) is used to identify patients who require NAC treatment following a POD based on their APAP concentration, time from ingestion and presence or absence of factors which increase risk of hepatotoxicity such as enzyme inducers or conditions predisposing to low hepatic glutathione stores. Patients with timed plasma concentrations above the appropriate treatment receive treatment with the full 20.25hrs course of intravenous NAC. In patients presenting up to 8hrs after ingestion, the need for NAC treatment can be assessed once the plasma APAP concentration is known. In patients presenting later than 8h as well as staggered POD, NAC treatment is initiated if the patient has ingested greater than 12 g (or 150 mg/kg) paracetamol or,

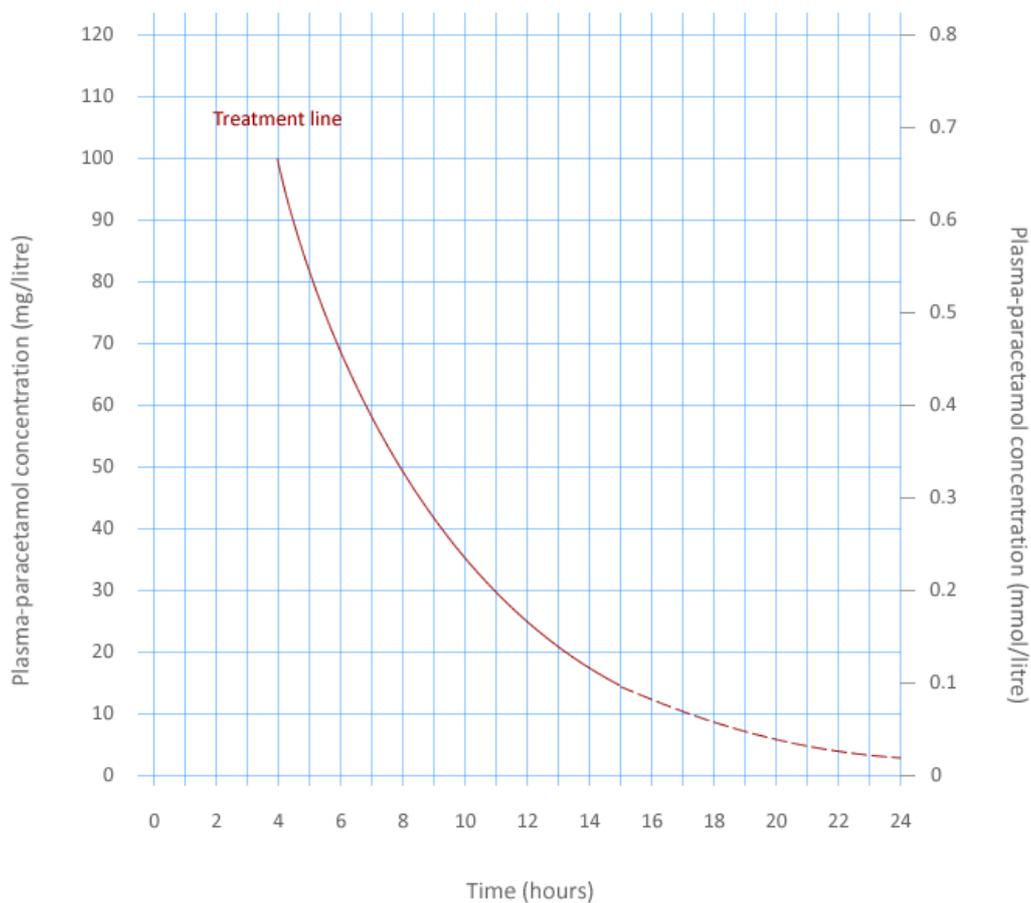
if they have risk factors, greater than 7.5 g. The need for continuation of NAC is then based on the plasma APAP concentration.

At the end of 20.25hr NAC regimen the decision to continue NAC is made by:

- the ALT has more than doubled since the admission measurement, OR
- the ALT is two times the upper limit of normal or more, OR
- the INR is greater than 1.3 (in the absence of another cause, e.g. warfarin)
- paracetamol/acetaminophen concentration greater than 20 mg/mL

Those that meet these criteria will continue to receive further NAC infusions at the concentration of the 16-hourly infusion until these blood results improve.

Figure 1: UK Paracetamol Overdose Treatment Nomogram



Recent studies in the Edinburgh and Newcastle-upon-Tyne toxicology units show that following the 20.25h NAC treatment where the loading dose is infused during 15 min, 51 of 346 (14.7%) patients had evidence of hepatocellular injury as measured by a 50% increase in their ALT after admission. Of these 51 patients, only 8 subsequently developed overt evidence of hepatocellular dysfunction as measured by an INR>2.0. All patients who subsequently developed an INR>2.0 had at least a 50% increase in their admission ALT after NAC treatment.

In a randomised controlled study, the incidence of anaphylactoid adverse drug reactions was 18% and 14% in patients receiving the initial loading dose of 150 mg/kg NAC over 15 minutes and 60 minutes respectively (Kerr et al 2005). While this study lacked the statistical power necessary to exclude clinically important benefits from the slower initial infusion rate, the results suggest that the slower initial infusion rate should be considered. In the United States, the FDA advised in 2006 that the initial loading dose of NAC be given over 60 minutes.

3.2 Studies in Overdose Patients

Patients with overdose are a challenging group to study. Treatment decisions need to be made quickly and, as they present with self-harm behaviour, consenting them to clinical study participation has been seen as potentially difficult.

Work both in Edinburgh and overseas suggests (Eddleston et al 2008 and Pakravan et al 2008), however, that these obstacles can be overcome and it is with this background that we developed this protocol to examine a new treatment approach to the most common poisoning seen in the UK.

The 2-phase 12hr acetylcysteine infusion protocol (100 mg/kg over 2hrs: 200 mg/kg over 10hrs) was studied in a formal factorial design against the traditional 3-phase 20.25hrs infusion protocol. The 12hr regimen was associated with very significant reductions in anaphylactoid reactions and vomiting compared with the 20.25hr infusion protocol.

The 12hr acetylcysteine protocol offers clinicians and patients the possibility for better targeting of therapy, fewer adverse effects, a simpler dosing regimen, and shorter hospital stay (Bateman et al 2014).

With the standard intravenous NAC regime, adverse reactions generally occur during or soon after the loading dose, leading to discontinuation of NAC until the reaction subsides. Despite this, patients who have reactions to intravenous NAC do not appear to have worse clinical outcomes.

This study, with a new treatment regime using PP100-01 co-treatment with the 12hr NAC regime, is designed to examine safety, tolerability and explore new experimental biomarkers in serum/plasma such as CK18 and microRNA (MiR122), GLDH and mitochondrial DNA to find new markers that identify organ injury earlier in the disease process than current markers.

3.3 Rational for the Study Drug

A completed non-clinical study in mice investigated the protective effect of PP100-01 (calmangafodipir) on POD and whether or not it was effective during the later oxidative phase of APAP-induced toxicity. The results support that PP100-01 offers long lasting protection against APAP induced ALT release in mice at a time-point when NAC no longer gives any protection.

PP100-01 has alone and in combination with NAC shown liver protective effects in mice at different time-points from 1hr to 6hrs after APAP intoxication, which can be translated to between 8 to 72hrs after APAP intake in humans (Figure 2).

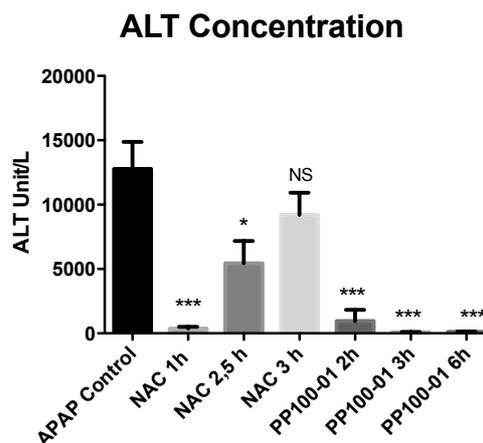


Figure 2. ALT concentrations in mice after POD and treatment with NAC (300 mg/kg) or PP100-01 (10 mg/kg) at different time-points.

3.4 Rational for the Dose in the Clinical Study

In the PLIANT study calmangafodipir (PledOx[®]) corresponding to 2, 5 or 10 µmol/kg was infused intravenously as a pre-treatment single dose over approximately 5 minutes. In this study there was a tendency for lesser effect on hematological parameters for the 10 µmol/kg dose in Part 1 (dose-escalation) of the PLIANT study therefore this dose was lowered to 5 µmol/kg. The better

effect of the low doses on the hematologic parameters is confirmed with the data from Part 2 of the PLIANT study.

PledOx[®] was well tolerated across all three doses 2, 5 and 10 µmol/kg calmangafodipir. PledOx[®] demonstrated an excellent safety profile with no meaningful differences between PledOx[®] treated arms and placebo in terms of adverse events.

During the treatment period, the PLIANT study showed a clinically relevant reduction in the incidence of sensory nerve damage (neuropathy) compared to placebo, and reduced the frequency, onset and duration of grade 2 or worse neuropathy compared with placebo, with a 43% reduction using 5 µmol/kg (OR 0,574 p=0,146). In the follow-up period (12 months including 4 visit each 3 month) a statistically significant persistent treatment effect on neuropathy is seen of the 5 µmol/kg PledOx[®] at the 3 and 6 month follow-up visit.

The 2 µmol/kg appeared less effective in reducing the frequency and onset of grade 2 or worse neuropathies (Sanofi-NCI).

At the proposed clinical dose levels (2, 5 and 10 µmol/kg calmangafodipir), there should be no safety issue with the exposure to either manganese, calcium or fodipir. The safety margin from exposure in toxicology studies are at least 30 times the highest proposed clinical dose of 10 µmol/kg calmangafodipir.

4 OBJECTIVES AND ENDPOINTS

The present study is designed to determine safety and tolerability of PP100-01 (drug substance, calmagafodipir) when administered as an add-on to the 12hr NAC treatment regime. The purpose of this study is to investigate a therapeutic regimen for POD where NAC is combined with the SOD mimetic, PP100-01 in order to evaluate if reduction of the oxidative stress on the liver will be safe and well tolerated. Furthermore, the study will assess experimental biomarkers in serum/plasma such as CK18 and microRNA (MiR122), GLDH and mitochondrial DNA to find new markers that track organ injury and therefore drug efficacy.

Primary objective:

Safety and tolerability of PP100-01 add-on treatment to the 12hr NAC treatment regime in patients treated for paracetamol/acetaminophen overdose (POD) when NAC treatment is initiated before 24hrs post POD.

Secondary objective:

Determine if there is evidence of PP100-01 having efficacy with regard to treatment of paracetamol-induced liver injury by measurement of conventional clinical biomarkers and novel experimental biomarkers.

Primary outcome

Adverse events and serious adverse events.

Secondary outcomes:

Clinical observations (pulse rate, blood pressure, respiratory rate, pulse oximetry, temperature)
Haematology and clinical biochemistry parameters

Experimental biomarkers in serum/plasma such as CK18 and microRNA (MiR122), GLDH and mitochondrial DNA

Incidence of hepatotoxicity between 12hr NAC arm and individual PP100-01 + NAC treatment arms
Duration of hospital stay (days for the individual PP100-01 + NAC arms vs 12hr NAC arm)

There will be no follow-up visit, but patients will be followed up using their electronic records. The following data is collected 7, 30 and 90 days after randomisation: Representation to hospital (any reason), representation with liver injury, repeat overdose, death and transfer to liver transplantation unit. Any AEs or SAES identified would be recorded and reported.

Exploratory secondary outcomes will be collected to improve the design of future clinical studies:

- To determine the rate of occurrence of hepatotoxicity (defined by raised biochemical markers) in patients treated with PP100-01 and 12hr NAC administration regimens.
- To compare the incidence of anaphylactoid reactions in the co-treatment and 12hr NAC regimens in APAP poisoned patients.
- To determine the occurrence of hepatotoxicity as determined at the end of the 12hr NAC administration regimen.
- To measure length of hospital stay in patients receiving co-treatment and 12hr NAC treatment regimens.
- Proportion of patients with a 50% increase in ALT after 10h post-treatment with NAC, compared with the admission value
- Proportion of patients with ALT > 100 at 10h post-treatment with NAC
- Proportion of patients with INR > 1.3 at 10h post-treatment with NAC
- Proportion of patients with paracetamol/acetaminophen concentration > 20 mg/mL

5 STUDY DESIGN

The study will be an open label explorative, randomised, dose rising design, NAC controlled study in patients treated for paracetamol/acetaminophen overdose using the 12hr NAC regime.

Entry into the study will depend on the patient's blood results confirming need for NAC.

Patients that have signed the informed consent will be treated with NAC followed by rising doses of the PP100-01. Each PP100-01 treatment dosing cohort will have 8 patients, 6 randomised to PP100-01 and 2 randomised to NAC alone.

- Group A: PP100-01 (2 µmol/kg calmangafodipir) after the "loading" dose of NAC
- Group B: PP100-01 (5 µmol/kg calmangafodipir) after the "loading" dose of NAC
- Group C: PP100-01 (10 µmol/kg calmangafodipir) after the "loading" dose of NAC

PP100-01 treatment is administered intravenously during 5 minutes.

The standard NAC regime will be continued with the second dose: 200 mg/kg NAC in 1000 ml i.v. over 10hrs.

The patients will continue NAC treatment according to standardised guidelines throughout the study until the patient is considered normalized and can leave the hospital.

The study will primarily evaluate safety and tolerability for treatment with PP100-01 in combination with NAC compared to the 12hr NAC treatment regime.

6 STUDY POPULATION

6.1 Number of Participants

We aim to recruit a total of 24 patients presenting with POD and requiring treatment with NAC to the study. We approximate this can be completed in a 6-12 months recruitment period.

6.2 Inclusion Criteria

1. Any patient with capacity admitted to hospital within 24 hrs either a single acute POD or more than one dose of paracetamol (staggered) and deemed to require treatment with NAC.
2. Provision of written informed consent
3. Males and females of at least 16 years of age

These patients will include:

- a) Patients presenting within 8h of overdose who have a timed blood paracetamol concentration above the 100-line on the UK APAP overdose treatment nomogram (See Figure 1).
- b) Patients presenting later than 8h who are at risk of liver injury from the reported dose ingested and have blood results confirming need for NAC
- c) Patients presenting after taking a staggered APAP overdose (defined as when the overdose of APAP is taken over a period of more than 1hr)

6.3 Exclusion Criteria

In order to participate in the study patients must not meet any of the following exclusion criteria:

1. Patients that do not have the capacity to consent to participate in the study
2. Patients detained under the Mental Health Act or deemed unfit by the Investigator to participate due to mental health.
3. Patients with known permanent cognitive impairment
4. Patients who are pregnant or nursing
5. Patients who have previously participated in the study
6. Unreliable history of POD
7. Patients presenting after 24hrs of POD
8. Patients who take anticoagulants (e.g. warfarin) therapeutically or have taken an overdose of anticoagulants

9. Patients who, in the opinion of the responsible clinician/nurse, are unlikely to complete the full course of NAC e.g. expressing wish to self-discharge
10. Prisoners
11. Non-English speaking patients. (Study information material will only be produced in English in view of the known and stable demographic of the Edinburgh self-harm population).

6.4 Participant Selection and Enrolment

6.4.1 Identifying Participants

Potentially eligible patients will be identified by treating clinicians at the Emergency Department of the Royal Infirmary of Edinburgh. They will inform an appropriately trained member of staff who will assess the patient for study eligibility. The Investigator or/and the study staff members who explains the study will ensure that the interview takes place without interruption and is responsible that the information is understood. The patient is encouraged to take time for reflection on the information presented before making a decision as to whether they wish to participate in the clinical study.

6.4.2 Consenting Participants

The patient will be given a Patient Information Sheet (PIS), which will explain the aims of the study and the potential risks and benefits of the study treatments.

The oral information will contain information on any foreseeable risks, side effects, complications and disadvantages, and that there may be unpredictable risks and discomforts associated with participation in a clinical study. The information shall also include information on any treatment that can be offered, if the patient chooses not to participate in the study. This information will also be given to the patient in writing in the Patient Information Sheet document.

The patient will be given enough time to consider the study and ask questions regarding their participation in the study. At most this could be about an hour but may be only 10-15 mins if the time lapse since APAP ingestion indicates a need to start treatment quickly.

If the patient wishes to participate in the study then the patient must sign the Informed Consent Form (ICF) before inclusion in the study. It is the Investigator's responsibility to ensure consent is obtained before any study specific procedures are carried out. Signing of the ICF occurs after the information has been received from both oral and written PIS. Both the patient and the person delegated to take consent will sign and personally date the ICF. The original signed ICF must be kept by the Investigator in the ISF, 1 copy is provided to the participant and 1 copy is placed in the medical file.

6.4.3 Assess Capacity

Capacity will be assessed by a doctor in the Emergency Department who has:

- completed GCP training (including specific training in assessing capacity)
- completed study specific training
- has been delegated this responsibility by the Principal Investigator

Only patients with capacity will be invited to participate in the study.

6.5 Screening for Eligibility

Patients where a clinical decision has been made to treat with NAC will be screened for eligibility to participate in the study and recorded on the screening log. The decision to treat with NAC will be made by the treating clinician based on protocols agreed by the National Poisons Information Service and available on TOXBASE, a web-based poisons information resource which is the UK national standard for therapy. Ineligible and non-recruited participants will be recorded on the screening log with a reason given.

At screening, age, sex and race will be recorded in the CRF for all patients.

All patients deemed eligible to take part in this study will be entered onto a Consent & Subject Status Log after consent and pre-screening checks are completed.

6.6 Treatment Allocation

6.6.1 Treatment Allocation Procedure

Eligible patients will be allocated to treatment according to a rising dose design. Allocation of patients to each dose will need to be completed before allocation can start for the next dose. In each dosing cohort there will be 8 patients. 6 will receive PP100-01 and NAC and 2 NAC alone. There will be a randomisation process at entry to the study that will determine treatment allocation within dosing cohort.

- Group A: PP100-01 (2 µmol/kg calmagafodipir) after the "loading" dose of NAC
- Group B: PP100-01 (5 µmol/kg calmagafodipir) after the "loading" dose of NAC
- Group C: PP100-01 (10 µmol/kg calmagafodipir) after the "loading" dose of NAC

Once a patient is allocated a treatment number he/she will remain in the study and have all outcomes recorded, unless he/she specifically withdraws consent to have data stored.

All medical and nursing staff caring for the patient will know (open label) treatment allocation. PP100-01, specially prepared and packaged for clinical study purposes, will be prepared and labelled to patient allocation number. Stores of packs will be held within the hospital pharmacy and sufficient packs supplied to the Emergency Department as required.

Doses of NAC and PP100-01 are based on the patient's weight and therefore infusions need to be prepared individually for each patient. NAC will be prepared by the clinical team or a delegated member of the research team and PP100-01 by a delegated member of the research team according to the 'Site Instructions for handling the IMP'.

A period of 24 hours will be left between recruitment of the first participant allocated to PP100-01+NAC treatment in each dosing cohort and recruitment of the next participant to allow adequate time for early adverse events to be identified.

6.6.2 Randomisation

The allocation sequence for each dosing cohort will be created using computer-generated random numbers, using blocking to ensure the required 6:2 ratio of NAC+PP100-01:NAC alone. The randomisation list will be held centrally at the Edinburgh Clinical Trials Unit (ECTU) in order to conceal treatment allocations until these are implemented via the ECTU secure web-based randomisation system.

6.6.3 Blinding

This is an open label study without blinding.

6.6.4 Patient Withdrawal

Patients are free to withdraw from the study at any time. If they withdraw from the study, this will be recorded, and data collected to that time point may be used if the patient agrees and the data included in the final analysis. If they withdraw consent to have their data stored, then they will be documented on the study Consort flow diagram as "withdrawn" and their data will not be used in the final analyses. Reasons for withdrawal will be collected if the patient is willing for us to do so, but they do not have to give a reason.

6.7 Patient Replacement

Replacement of patients will be allowed in order to obtain the planned number of evaluable patients in each dosing cohort. Any patients that discontinue treatment for a reason other than Dose Limiting Toxicity (DLT) will be replaced.

7 PATIENT ASSESSMENTS AND DATA COLLECTION

7.1 Description of Assessments

7.1.1 Blood Samples

NAC will be given to all patients arriving to the hospital according to the standard guidelines when paracetamol/acetaminophen overdose is suspected. All patients that arrive to the hospital before 24hrs after POD will be asked if they will participate in the study.

As part of routine clinical care all patients presenting to hospital following a paracetamol overdose will have the following blood tests on arrival to hospital or 4hrs after overdose (whichever is the longer time).

- S-APAP level in serum
- Urea, sodium, potassium and creatinine
- Liver function tests (LFTs) ALT, Bilirubin and ALP
- Clotting screen: INR and Prothrombin time (PT)
- Haemoglobin, White Blood Cells(WBC), Mean Cell Volume (MCV)

Immediately prior to administering PP100-01 a study specific blood sample will be collected (10mL split over serum and plasma) which will measure:

- ALT, bilirubin and alkaline phosphatase (ALP)
- Prothrombin time and INR
- Serum creatinine
- Biomarkers: CK18 and microRNA MiR122, GLDH, mitochondrial DNA and others
- PP100-01 levels

These measurements are part of the sub-study examining novel biomarkers.

As part of routine clinical care there will be blood sampling at 10 hrs and 20 hrs after starting NAC. The following are routinely measured:

- S-APAP level in serum
- Urea, sodium, potassium and creatinine
- Liver function tests (LFTs) ALT, Bilirubin and ALP
- Clotting screen: INR and Prothrombin time (PT)
- Haemoglobin, White Blood Cells(WBC), Mean Cell Volume (MCV)

At these 10hr and 20hr venepuncture episodes study specific blood samples will also be collected (at each time 10mL split over serum and plasma) which will measure:

- Biomarkers: CK18 and microRNA MiR122, GLDH, mitochondrial DNA and others
- PP100-01 levels

7.1.2 Medical history and concomitant diseases

A complete review of the patient's past medical history will be conducted. Any concomitant disease, whether considered relevant for the study or not by the Investigator, must be recorded in the CRF.

7.1.3 Vital signs

Vital signs will be assessed at baseline (between 1 and 2 hrs after starting NAC) and again at 2.5, 10 and 20hrs after starting NAC. This will be recorded in the Case Report Form (CRF). The following vital signs will be evaluated in a supine position after at least 5 minutes of rest: blood pressure- systolic and diastolic (mmHg), heart rate (BPM), respiration rate (/min), pulse oximetry, temperature (°C) and temperature route.

Height (estimated) and weight will be collected at baseline.

7.1.4 Physical examination

A baseline physical exam will be completed assessing the Cardiovascular, Respiratory and GI systems and this data recorded on the CRF. Any AEs/SAEs identified during the exam will be recorded/reported. Additional full physical examinations will be completed if AE/SAEs are reported, requiring a more thorough exam to be carried out. This will include assessment of local toxicity at the site of intravenous administration.

All new findings or changes to previous findings from vital signs or baseline physical exam considered clinically significant are to be recorded in the CRF, as an adverse event if the finding is made subsequent to the patient signing the ICF for the main study (See Section 11). If the patient has received a dose of study medication it should be deemed whether the AE is related or non-related to the study drug.

7.1.5 Pregnancy test

All women of childbearing potential must be screened for pregnancy by urine or serum hCG test as part of the eligibility assessment.

7.1.6 ECG

An ECG will be performed on resting patients at baseline, 2.5hr, 10hr and 20hr and any clinically significant deviations recorded.

7.1.7 Treatment Phase

Treatment will start with the first NAC bag of 100 mg/kg in 200 ml ("loading dose") at time point '0'. Immediately prior to PP100-01 a study specific blood sample will be collected (time 2hr).

PP100-01 treatment is administered intravenously as a bolus infusion over 5 minutes at the dose specified by the dosing cohort:

- Group A: PP100-01 (2 µmol/kg calmangafodipir) after the "loading" dose of NAC
- Group B: PP100-01 (5 µmol/kg calmangafodipir) after the "loading" dose of NAC
- Group C: PP100-01 (10 µmol/kg calmangafodipir) after the "loading" dose of NAC

All necessary precautions will be taken during administration of the study drug. The patient will be monitored with vital signs (Section 7.1.3). Observation and medical treatment for anaphylactic shock will, as a standard be requisite.

The 12hr NAC regime will be continued with the second dose: 200 mg/kg NAC in 1000 ml i.v. over 10 hr. As per routine clinical practice, there will be one blood sample taken 2 hrs before the end of the second NAC bag (the 10hr time-point) and a second blood sample taken 10h later (the 20hr time-point).

The following procedures should be performed during the treatment phase at time-points: baseline, 2hr, 10hr and 20hr (see study schedule, section 7.2 (Table 1)):

- ALT, bilirubin and alkaline phosphatase (ALP)

-
- Prothrombin time and INR
 - Serum creatinine
 - Biomarkers: CK18 and microRNA MiR122, GLDH, mitochondrial DNA and others
 - PP100-01 level will be assessed at 2hr, 10hr and 20hr

Any rescue medication given will be recorded in the Case Report Form by routine clinical staff.

7.1.8 End of treatment

At the end of the 12hr NAC regimen the decision to continue NAC is made by assessment of the blood sample taken at the 10hr time-point:

- the ALT has more than doubled since the admission measurement, OR
- the ALT is two times the upper limit of normal or more, OR
- the INR is greater than 1.3 (in the absence of another cause, e.g. warfarin)
- paracetamol/acetaminophen concentration > 20 mg/mL

7.1.9 Adverse events

Adverse events/ adverse reactions will be assessed at baseline, 2hr, 2.5hr 10hr, 20hr and 22hr and as part of follow up (section 7.1.10). A full physical exam will be carried out if AEs/SAEs are identified that require a more thorough exam to be performed and this will be recorded on the CRF.

Episodes and incidence of vomiting and retching will be recorded objectively by nursing staff on the AE log and reported as an SAE if classified as serious.

Adverse events will be recorded and reported as detailed in Section 11.

7.1.10 Follow-up

There will be no follow-up visit, but patients will be followed up using their electronic records. The following data is collected 7, 30 and 90 days after randomisation: Representation to hospital (any reason), representation with liver injury, repeat overdose, death and transfer to liver transplantation unit. Any AEs or SAEs identified will be recorded and reported.

7.1.11 Assessment timelines

2, 2.5 hour assessments should be performed +/-15mins of the timepoint. PP100-01 treatment should be +/- 10mins of timepoint. 10, 20 hour assessments should be performed +/-30minutes of the timepoint.

7.2 Table of Assessments

Visit windows	Screen ing	0 (NAC treatment)	Baseline (Pre-PP100-01)	After 2 hrs	After 2.5 hrs	After 10 hrs	After 12 hrs	After 20 hrs	Discharge after 22 hrs	7 day follow up	30 day follow up	90 day follow up
Informed Consent	X											
Eligibility Criteria	X											
Enroled into trial	X											
Treatment Allocation/randomisation	X											
Bloods												
APAP conc level	X					X		X				
INR /Prothrombin	X			X		X		X				
ALT (IU/L)	X			X		X		X				
ALP (IU/L)	X			X		X		X				
Bilirubin	X			X		X		X				
Creatinine	X			X		X		X				
Hb	X					X		X				
Urea	X					X		X				
Sodium	X					X		X				
Potassium	X					X		X				
MCV	X					X		X				
WBC	X					X		X				
Inflammation Biomarkers				X		X		X				
PP100-01 levels				X		X		X				
Pregnancy test	X											
Treatment												
NAC Treatment		X			X		(X)	(X)				
PP100-01 Treatment				X								
Physical Examination			X	X*	X*	X*		X*				
ECG			X		X	X		X				
Vital signs (BP and P)			X		X	X		X				
Adverse events			X	X	X	X		X	X	X	X	X

*If AEs/SAEs identified that require a full physical exam to be completed
(x) NAC continued as per TOXBASE (12hours +/-30mins).
2, 2.5 hour assessments should be performed +/-15mins of the timepoint. PP100-01 treatment should be +/- 10mins of timepoint. 10, 20 hour assessments should be performed +/-30minutes of the timepoint.

8 STUDY MEDICATION

8.1 Study Medication 1, PP100-01

PP100-01, calmagafodipir in concentration 50 mM (corresponding to 10 mM Mn II) is an aseptically prepared, sterile filtered product. PP100-01 is provided as a sterile, bright yellow clear solution in 20 ml single dose glass vials. The pH is adjusted between 7.4 and 7.6 with sodium hydroxide to protect against hydrolysis.

8.1.1 Receipt of PP100-01

The active ingredient, calmagafodipir, is manufactured at Albany Molecular, Research Inc. (AMRI), Albany NY, USA. The final IMP (PP100-01) will be manufactured, packed and labelled at Recipharm Pharmaceutical Development AB, Solna Sweden.

PP100-01 will be supplied to each site already packaged and labelled. IMP will be shipped to the local depot to be further sent to the hospital pharmacy or directly to the hospital pharmacy. All used and unused IMP must be kept at the site for accountability purposes.

8.1.2 Packaging and labelling of PP100-01

PP100-01 is packaged in kits each containing solution in 20 ml glass vials that are to be stored and transported frozen.

The glass vials will be labelled in English.

8.1.3 Storage and handling

PP100-01 should be stored in a safe place with limited access. PP100-01 is to be protected from light and stored at -15 to -25°C. Maintenance of a temperature log is mandatory. The log should be updated by delegated site personnel twice per week.

PP100-01 should be thawed by holding it in a hand for at least 5 minutes to defrost it. The thawed vial is stable when stored in refrigerator for up to 6 days prior to use. PP100-01 may be thawed and refrozen once, but this should be avoided. Temperature logs must be kept for the freezer and refrigerator where the PP100-01 is stored. The temperature should be noted twice weekly (unless automatic temperature readings are available).

8.1.4 PP100-01 accountability

It is the responsibility of the Investigator to establish a system for handling the PP100-01, to ensure that:

- Deliveries of such products are correctly received and recorded by a designated person
- PP100-01 is handled and stored safely and properly
- PP100-01 is given only to study patients in accordance with the CSP
- All unused PP100-01 and empty containers are stored until they have been checked by the monitor
- It is possible to reconcile records of all used and unused stocks as confirmed by Investigator's signature

The monitors should account for all PP100-01 at the study site. Used PP100-01 vials will be accounted and disposed at the sites. Unused PP100-01 vials should be kept and sent for destruction.

8.1.5 Assessment of PP100-01 compliance

Assessment of patient compliance is not applicable as the PP100-01 is administered at the clinic by a study nurse as an infusion. The investigator has the responsibility to document all PP100-01 dispensed in the accountability log and in the medical records.

8.1.6 Procedure for preparation of patient dose:

Doses of PP100-01 are based on the patient's weight and therefore infusions need to be prepared individually for each patient. At the study site, the PP100-01 should be prepared immediately before each patient administration according to procedure for preparation of the PP100-01.

Any unused Product should be destroyed according to local requirements and regulations. The PP100-01 will be supplied to the clinical study sites as a 50 mM calmagafodipir concentrate for solution for intravenous infusion.

The preparation process will be performed as described below and should be repeated before each administration:

1. Patient is weighed and the weight is documented in the patients CRF.
2. Based on the weight of the patient and the current applicable dose step, the dose is calculated:
 - 2 µmol/kg calmagafodipir dose will be 0.04 mL/kg
 - 5 µmol/kg calmagafodipir dose will be 0.10 mL/kg)
 - 10 µmol/kg calmagafodipir dose will be 0.20 mL/kg)
3. Dose will be documented in the patients CRF.
The research staff will prepare PP100-01 solution with a dose as calculated in point 2 above, and record the volume. This solution is made up to a final volume of 20mL with normal saline. Volume over 20mL will not require the addition of normal saline. The solution is aspirated into a suitable syringe. The PP100-01 solution in the syringe is stable for 3 hours in ambient conditions and 6 hours when refrigerated.

8.1.7 Investigator's Brochure

The Investigator's Brochure is given in Appendix A.

8.2 Study Medication 2, N-acetylcysteine

8.2.1 Study Drug Identification

Acetylcysteine, trade name of Parvolex

8.2.2 Study Drug Manufacturer and Marketing Authorisation Holder

UCB Pharma Limited
208 Bath Road
Slough
Berkshire
SL1 3WE

Marketing Authorisation: PL 00039/0410

8.2.3 Labelling and Packaging

Acetylcysteine for use in the study will be available as 10 mL ampoules containing 200 mg/mL acetylcysteine.

The acetylcysteine will be taken from existing hospital stock.

8.2.4 Storage

Acetylcysteine ampoules will be stored in a cupboard at the study site.

8.2.5 Summary of Product Characteristics

The Summary of Product Characteristics (SoPC) is given in Appendix B

9 DOSAGE AND ADMINISTRATION

9.1 Dosing Regime Acetylcysteine (NAC)

Standard NAC regimen:

300 mg/kg NAC IV (200 mg/mL) in 5% glucose (dextrose) or 0.9% Sodium chloride over 12hr: 100 mg/kg in 200 mL over 2 hr ("loading dose"), then 200 mg/kg in 1000 mL over 10hr.

Note that for patients weighing more than 110 kg, the dose will be calculated using a 110 kg weight as per the TOXBASE guidance.

9.2 Dosing of PP100-01

- Group A: PP100-01 (2 µmol/kg calmangafodipir) after the "loading" dose of NAC
- Group B: PP100-01 (5 µmol/kg calmangafodipir) after the "loading" dose of NAC
- Group C: PP100-01 (10 µmol/kg calmangafodipir) after the "loading" dose of NAC

9.3 Dose Changes

Dose changes will not be allowed unless patients develop anaphylactoid reactions. These will be managed conventionally with temporary discontinuation of the infusion, symptomatic treatment as required and restarting at a lower rate once the reaction has subsided.

9.4 Participant Compliance

The study drugs will be administered by nursing staff either as a single intravenous bolus or as an infusion. Treatment will be supervised in hospital by a clinician.

9.5 Overdose

It is not expected that overdose with study drugs will occur. Total doses are contained within the study framework. The clinicians managing the study are clinical toxicologists.

9.6 Other Medications

Other than the study drugs and paracetamol, there are no additional prohibited medications. Rescue medication for nausea or vomiting is permitted. The protocol advocates the use of intravenous cyclizine in these circumstances. Any rescue medications should be recorded in the CRF.

Any medication (prescription as well as over the counter (OTC) drugs) or therapeutic intervention deemed necessary for the patient, and which, in the opinion of the Investigator, do not interfere with the safety and efficacy evaluations, may be continued. However, the Investigator should be cautious in evaluating the need for change in dosage and should carefully assess if any concomitant medication is necessary. If possible, all unnecessary concomitant medication should be stopped before entering the patient into this study. Concomitant medications received at least within 30 days prior to the patient providing written informed consent for the main study should be recorded.

9.7 Study Medication Accountability

The Investigator or designee is responsible for maintaining accountability records for all inventory transactions (i.e. receipt and return). Only personnel authorised by the PI should handle and administer the study medication. The study team must complete and return the study medication supply form to the Sponsor, verifying the receipt of study medication.

10 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

10.1 Adverse Events

Participants will be instructed to alert the study team at any time after consenting to join the study, until discharge if any symptoms develop. All adverse events (AEs) identified must be recorded in detail in the CRF. If the patient has received a dose of study medication it should be deemed whether the AE is related or non-related to the study drug. Any AEs identified as part of the 7, 30 and 90 day follow up will be recorded/reported.

The Investigator is responsible for the detection and documentation of events meeting the criteria and definitions detailed below. In the case of an AE, the Investigator will initiate the appropriate treatment according to their medical judgment. Participants with AEs present at hospital discharge must be followed up until resolution of the event.

Full details of contraindications and side effects that have been reported following administration of NAC can be found in the relevant Summary of Product Characteristics (SoPC) in Appendix B.

10.2 Definitions

An **adverse event** (AE) is any untoward medical occurrence in a clinical study patient who is administered a medicinal product, which does not necessarily have a causal relationship with the treatment.

An **adverse reaction** (AR) is any untoward or unintended response to an investigational medicinal product related to any dose administered.

An **unexpected adverse reaction** (UAR) is an adverse reaction that is not consistent with the product information in the IB/SoPC.

A **serious adverse event** (SAE), **serious adverse reaction** (SAR) or **suspected unexpected serious adverse reaction** (SUSAR) is any AE, AR or UAR that at any dose:

- results in death;
- is life threatening (i.e. the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- requires hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect.

Note: Hospitalisations for treatment planned prior to randomisation and hospitalisation for elective treatment of a pre-existing condition will not be considered as an AE. Complications occurring during such hospitalisation will be AEs.

10.3 Detecting AEs and SAEs

All AEs and SAEs must be recorded from the time a participant consents to join the study until hospital discharge.

The Investigator should ask about the occurrence of AEs/SAEs during the infusion and until hospital discharge. Open-ended and non-leading verbal questioning of the participant should be used to enquire about AE/SAE occurrence. If there is any doubt as to whether a clinical observation is an AE, the event should be recorded.

Patient electronic records will be reviewed at 7 days, 30 and 90 days after discharge for AEs and SAEs.

10.4 Recording AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. The Investigator should then record all relevant information in the AE log and on the SAE form (if the AE meets the criteria of serious).

Information to be collected includes dose, type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

10.4.1 Assessment of Seriousness

The Investigator should make an assessment of seriousness as defined in Section 10.2.

10.4.2 Assessment of Causality

The Investigator must make an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions. All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g. possibly, probably, definitely) to the study drug will be considered as ARs/SARs. If concomitant or rescue/escape drugs are given, the Investigator must also make an assessment of whether the AE/SAE is likely to be related to an interaction between the study drug and concomitant or rescue/escape drugs or whether the AE/SAE might be linked to either the study drug or concomitant or rescue/escape drugs but cannot be attributed to only one of these drugs. All AEs/SAEs judged as being related (e.g. possibly, probably, definitely) to an

interaction between the study drug and concomitant or rescue/escape drugs, or any AE/SAE that cannot be attributed to only the study drug or the concomitant or rescue/escape drugs will also be considered to be ARs/SARs.

Unrelated: where an event is not considered to be related to the study drug.

Possibly: although a relationship to the study drug cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.

Probably: the temporal relationship and absence of a more likely explanation suggest the event could be related to the study drug.

Definitely: The known effects of the study drug or its therapeutic class, or based on challenge testing, suggest that study drug is the most likely cause. Alternative causes such as natural history of the underlying disease, other risk factors and the temporal relationship of the event to the treatment should be considered and investigated. The blind should not be broken for the purpose of making this assessment.

10.4.3 Assessment of Severity

The Investigator should make an assessment of severity for each AE/SAE and record this on the CRF according to one of the following categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: an event that prevents normal everyday activities.

Note: the term 'severe', used to describe the intensity, should not be confused with 'serious' which is a regulatory definition based on participant/event outcome or action criteria. For example, a headache may be severe but not serious, while a minor stroke is serious but may not be severe.

10.4.4 Assessment of Expectedness

If an event is judged to be an AR/SAR, the evaluation of expectedness should be made based on knowledge of the reaction and the relevant product information documented in the IB/SoPC.

10.5 Reporting of SAEs/SARs/SUSARs

Once the Investigator becomes aware that an SAE has occurred in a study participant, they must report the information to PRODUCTLIFE within 24 hrs. The SAE form must be completed as thoroughly as possible with all available details of the event, signed by the Investigator. If the Investigator does not have all information regarding an SAE, they should not wait for this additional information before notifying PRODUCTLIFE. The form can be updated when the additional information is received.

The SAE report must provide an assessment of causality and expectedness at the time of the initial report.

The SAE will be reported by PRODUCTLIFE Ltd, The Jeffreys Building, St John's Innovation Park, Cowley Road, Cambridge, CB4 0DS, United Kingdom, Tel: +44 (0) 1223 402 660, Fax: +44 (0) 1223 413 689, Web: www.productlife-group.com, E-mail: safety@productlife-group.com.

10.6 Regulatory Reporting Requirements

As soon as the Investigator is aware of a potential Serious Adverse Event (SAE), he/she should contact PRODUCTLIFE by telephone (+44 1223 402 660) or E-mail (safety@productlife-group.com) no later than 24 hours after the knowledge of such a case. Reporting of the SAE via E-mail must be done by E-mailing an SAE form to safety@productlife-group.com.

If identification of the event occurs outside of office hours, the SAE form must be sent to safety@productlife-group.com or faxed (+44 (0)1223 413689) where the PRODUCTLIFE safety team will become aware of it at the start of the next business day (8.30 – 17.00). Urgent out-of-

hours calls will be redirected to and qualified safety team member who will record the details of the call. The SAE must contain the minimum criteria for the case to be valid: an identifiable patient, suspect treatment/drug, adverse event and a reporter. Information regarding the causality should also be provided. The Investigator should follow-up the initial notification of the potential SAE by E-mailing a copy of the SAE reporting form to PRODUCTLIFE at the number provided in the Investigator Site File. The E-mailed SAE reporting form should be received at PRODUCTLIFE within 24 hours after knowledge of such a case.

A Development Safety Update Report will be submitted to the regulatory competent authority and the main REC listing all SARs and SUSARs.

10.7 Follow up Procedures

After initially recording an AE or recording and reporting an SAE, the Investigator is required to follow each participant until resolution. Follow up information on an SAE should be reported to PRODUCTLIFE.

Follow-up information on an existing SAE that is fatal or life-threatening should be reported by the Investigator to PRODUCTLIFE within 5 days after the initial report. Where appropriate, hospitalisation or autopsy reports should be made available. All serious adverse events will be followed up until resolution (i.e., asymptomatic, stabilisation or death).

AEs still present in participants at hospital discharge should be monitored until resolution of the event or until no longer medically indicated.

10.8 Reporting of Suspected Unexpected Serious Adverse Reactions by PRODUCTLIFE

Suspected unexpected serious adverse reactions (SUSARs) will be reported by PRODUCTLIFE according to appropriate Competent Authority and Ethics Committee requirements. Sponsor will report SUSARs to the Investigator in a blinded manner, following appropriate notification to the Competent Authorities and Ethics Committees. SUSAR reporting to the Competent Authorities and Ethics Committees will be performed according to local regulations in an unblinded manner. The Competent Authorities will be notified of all SUSARs through the Eudravigilance database.

Fatal and life-threatening SUSARs should be reported by PRODUCTLIFE as soon as possible to the Competent Authorities and Ethics Committees according to local regulations, and in any case no later than 7 calendar days after knowledge by PRODUCTLIFE of such a case. Relevant follow-up information on the case will be subsequently communicated within an additional 8 days. All other SUSARs shall be reported to the Competent Authorities concerned and to the Ethics Committee concerned according to local regulations as soon as possible but within a maximum of 15 days of first knowledge by PRODUCTLIFE.

10.9 Pregnancy Reporting

Pregnancy is an exclusion for participation and pregnancy does not need to be reported if it is discovered before taking any dose of the study drug. However if a participant becomes pregnant between discharge from hospitalisation and the 90-day follow-up period this should be recorded and reported with a view to obtaining outcome of the pregnancy.

The investigator must report the pregnancy within 24 hours of being notified of it by emailing the "Clinical Trial Pregnancy Form: Initial Report" to safety@productlife-group.com (or faxing if email not possible on 01223 413 689). PRODUCTLIFE will inform the Sponsor of the pregnancy within 1 working day of receiving notification. The investigator must diligently follow the participant until delivery or termination of pregnancy, providing necessary updated information to PRODUCTLIFE using the 'Clinical Trial Pregnancy Form: Follow-up Report'.

Information on the status of the mother and child after delivery will be forwarded to PRODUCTLIFE using the 'Pregnancy Outcome;' section of the 'Clinical Trial Pregnancy Form: Follow-up Report'. Generally follow up will occur within 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will also be reported on this form.

Although pregnancy occurring in a clinical study is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded

as an AE or SAE and will be followed up as such. A spontaneous abortion is always considered to be an SAE and should be recorded and reported to PRODUCTLIFE as such.

10.10 Independent Safety Data Monitoring Committee (SDMC)

An independent safety data monitoring committee (SDMC) will be appointed to review accumulating adverse event data and patient safety. The SDMC will evaluate the safety in relation to PP100-01 dosing step increase. The committee will give recommendations on the continuation or termination of the study by detection of any safety signals as early as possible in accordance with the SDMC Charter.

Proposed composition of SDMC will be: Chairperson, Hepatologist, Statistician, Independent Clinical Pharmacologist/Toxicologist.

During the period of recruitment into the study, interim analyses of in-hospital mortality/morbidity and of any other information that is available on major outcome events (including serious adverse events believed to be due to treatment) will be supplied, in strict confidence, to the Chairperson of the SDMC, along with any other analyses that the committee may request.

All further patient enrolment will be paused pending advice from the SDMC if one of the following stopping rules have been met: 1. Patient death, admission to a Critical Care Unit or admission to a Liver Transplantation Unit due to any reason, or 2. One SUSAR that definitely or probably relates to either PP100-01 or NAC or both.

All SDMC data review will be documented and all meetings will have written minutes, which will be filed in the Trial Master File (TMF) upon completion of the study.

11 DATA MANAGEMENT

Data collection

Data will be collected by trained and delegated members of the research team from routinely available NHS hospital records or trial specific documentation onto a paper CRF. The data will be transposed into an electronic CRF. A personal log-in will be provided for all responsible personnel to allow for an audit study relating to the study data to be maintained.

The trial data should be entered in a timely manner into the CRF by a member of the site staff delegated responsibility for this specific task by the Principal Investigators (PI) of the clinical site. It is the responsibility of the Investigator to ensure that the CRFs are properly completed. The data in the CRFs should be consistent with the relevant source documents. The Investigator will sign the designated signature fields of the CRF data entry screens to confirm that the information on each screen is accurate and complete. All data must be stored in an unidentifiable form treated with strict confidentiality in accordance with applicable data-protection regulations.

Captured data will be monitored electronically and Source Data Verification (SDV) will take place against the individual patient records unless the CRF is considered source data. Any inconsistencies will be presented as queries; either as automatically generated queries if raised by the logical data checks of the CRF system, or by manually generated queries if raised by the data validation checks or the SDV performed by the Monitor or Data Manager (DM). Queries shall be resolved in a timely manner by a trained member of the site staff.

12 STATISTICS

12.1 Statistical Analysis Plan

The principal features of the statistical analysis of the data are described in this section. A more technical and detailed elaboration of the principal features will be written in a separate Statistical Analysis Plan (SAP).

12.2 Analysis Data Sets

Full analysis population:

Patients will be included in the full analysis population, the primary population for analysis of efficacy, if they have received any PP100-01 or NAC. Data will be analysed according to the randomised treatment group.

Per protocol population:

The stringent per protocol population includes patients from the full analysis population for whom the study protocol has been followed without any major violations.

Safety population:

The population for safety analysis will be patients who have received any PP100-01 or NAC. Data will be analysed according to the treatment received (NAC plus PP100-01; or NAC alone). Any patient who withdraws during the treatment phase of the study will be included in the safety population (adverse events and laboratory parameters). Data for all patients will be listed, and a list of withdrawn patients, with all reasons for withdrawal, will be given.

Data will also be listed for those patients who, after having consented to participate, underwent baseline examinations required for inclusion into the study but who because a criterion for exclusion was met or for other reasons were not included in the study.

12.3 Estimation of Sample Size

With the clinical safety data that are available we do not expect any adverse events but we note that PP100-01 has not been administered in paracetamol overdose patients treated with NAC. We deem that 6 patients per group in this initial dose escalation study will allow initial exploration of effects on biomarkers and potential dose limiting toxicity.

12.4 Proposed Statistical Analysis

A CONSORT diagram depicting the flow of participants through the study will be reported. Descriptive statistics will be used to report baseline characteristics by treatment group and overall: continuous variables will be summarised by the mean, standard deviation, median minimum and maximum; categorical variables will be summarised using the number and percentage in each category. Log transformation will be used where appropriate.

We will keep missing data to an absolute minimum, but where there is missing data those records will be removed from the analysis; if missing data rates are substantial the effect of this will be investigated using sensitivity analyses.

Binary outcomes (including the primary outcome) will be reported by treatment group and overall using the proportion and exact 95% confidence interval. Binary outcomes will be compared within dosing cohort between the PP100-01+NAC and the NAC alone patients using a difference in proportions and its exact 95% confidence interval. Binary outcomes will be compared between each of PP100-01 dosing groups A, B and C and the combined NAC alone group in the same way.

Continuous outcomes will be reported by treatment group and overall using the mean and 95% confidence interval. Continuous outcomes will be compared within dosing cohort between the PP100-01+NAC and the NAC alone patients using the difference in means and its 95% confidence interval. Continuous outcomes will be compared between each of PP100-01 dose groups A, B and C and the combined NAC alone group in the same way. The continuous outcome analyses listed above will be repeated using the change from baseline in each continuous outcome.

13 ETHICAL CONSIDERATIONS

13.1 Benefit-Risk Assessment

The risks with the study treatment are unknown, since no previous studies have been performed in humans using PP100-01 co-treatment with NAC. The toxicological studies in animals show that PP100-01 is safe and well tolerated in doses more than 30 times the proposed highest PP100-01 dose in this study. Furthermore, the human clinical experience in the completed phase 2b study

show that calmagafodipir also is safe and well tolerated in human patients, (see section 2.3 and the Investigator Brochure (IB) for more information).

Thus, if balanced against the fact that the investigational product has demonstrated promising effects in preclinical studies and might have effect in POD patients the benefit-risk ratio is predominantly positive.

The need for treatment in this patient group far outweighs the risks and there is evidence to suggest that NAC is safe in pregnancy and single doses of PP100-01 are highly unlikely to be a teratogenic risk.

A defined recruitment strategy will be used in the emergency department to target all eligible patients.

Through this study, information on PP100-01 co-treatment with NAC effects in human will be established, which could result in further clinical studies, and hopefully market authorisation which would be beneficial to a large population, since the incidences of POD are high world-wide.

13.2 Informed Consent

It is the Investigator's responsibility to ensure consent is obtained (as detailed in section 6.4.2).

13.3 Ethics, Regulatory Authority and local approval.

The trial design and study documentation will be reviewed and approved by the Sponsor, REC, MHRA and local R&D prior to commencement of the study. Any amendments made during the study will be reviewed and approved prior to implementation. Annual progress reports will be submitted as required.

14 STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

14.1 Project Management Group

The study will be coordinated by a Project Management Group, consisting of the Principal Investigator in Edinburgh, ECTU Trial Manager and coordinating research nurse. The ECTU Trial Manager will oversee the study and will be accountable to the Principal Investigator. Any queries will be resolved by the Investigator or delegated member of the study team.

14.2 Inspection of Records

Investigators and institutions involved in the study will permit study related monitoring, audits, REC review, and regulatory inspection(s). In the event of an audit, the Investigator agrees to allow the Sponsor, representatives of the Sponsor or regulatory authorities direct access to all study records and source documentation.

14.3 Study Monitoring

The study will be monitored according to the Monitoring and SDV plan. The research team should keep the monitor informed about the progress of study recruitment in order to plan timed monitoring visits.

Prior to the start of the study, the designated monitor will review the protocol and CRF with the Investigator and his/her staff. The Investigator will be visited on a regular basis by the monitor, who will check study procedures and paperwork. The monitor must be allowed to review patient records to confirm that required protocol procedures are being followed and check consistency between patient record and CRF data. Incorrect or missing entries in the CRFs will be queried and must be corrected immediately.

15 GOOD CLINICAL PRACTICE

15.1 Ethical Conduct

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).

15.2 Regulatory Compliance

The study will not commence until a Clinical Trial Authorisation (CTA) is obtained from the appropriate Regulatory Authority. The protocol and study conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004, and any relevant amendments.

15.3 Investigator Responsibilities

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff. Delegated tasks must be documented on a Delegation Log and signed by all those named on the list.

15.3.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out as detailed in section 6.4.2.

15.3.2 Study Site Staff

The Investigator must be familiar with the PP100-01, protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the PP100-01, protocol and their study related duties.

15.3.3 Data Recording

The Investigator is responsible for the quality of the data recorded in the CRF.

15.3.4 Investigator Documentation

Prior to beginning the study, each Investigator will be asked to provide particular essential documents for the TMF:

- An original signed Investigator's Declaration (as part of the Clinical Study Agreement documents);
- Curriculum vitae (CV) signed and dated by the Investigator indicating that it is accurate and current.

15.3.5 GCP Training

All study staff must hold evidence of appropriate GCP training or undergo GCP training. This should be updated every two years throughout the study and a copy retained in the TMF and ISF.

15.3.6 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain participant confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee, Regulatory Authorities, or the REC. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

15.3.7 Data Protection

All Investigators and study site staff involved with this study will comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to those clinicians treating the participants.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

15.4 Monitoring / Quality Control

The Investigator should facilitate with the monitoring of the study (See section 15.3).

15.5 Quality Assurance

During or after the study is completed, sponsor representatives or regulatory authorities may wish to carry out an audit or an inspection. These representatives must have the same access to study data and patient source data as the monitor.

16 STUDY CONDUCT RESPONSIBILITIES

16.1 Protocol Amendments

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant, must be reviewed and approved by the Principal Investigator.

Amendments to the protocol must be submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to participants being enrolled into an amended protocol.

16.2 Protocol Violations and Deviations

Investigators should not implement any deviation from the protocol without agreement from the Principal Investigator and appropriate REC, Regulatory Authority and R&D approval except where necessary to eliminate an immediate hazard to study participants.

In the event that an Investigator needs to deviate from the protocol, the nature of and reasons for the deviation should be recorded in the CRF. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval if appropriate.

Protocol deviations and violations should be recorded on the appropriate forms and submitted to the Sponsor within 1 week for deviations, within 3 days for violations.

16.3 Study Record Retention

All study documentation will be kept for at least 5 years in accordance with the Sponsor recommendations.

16.4 Serious Breach Requirements

A serious breach is a breach which is likely to effect to a significant degree:

- (a) the safety or physical or mental integrity of the participants of the trial; or
- (b) the scientific value of the trial.

If a potential serious breach is identified by the Principal Investigator or delegates, the Sponsor must be notified within 24hrs. It is the responsibility of the Sponsor to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and report to regulatory authorities and REC as necessary.

16.5 End of Study

The end of study is defined as when the last participant's 90-day follow up is completed.

The Investigators have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC and Regulatory Authority within 90 days, or 15 days if the study is terminated prematurely. A summary report of the study will be provided to the REC and Regulatory Authority within 1 year of the end of the study.

16.6 Continuation of Drug Following the End of Study

In the event of the need for further NAC treatment during this admission as a result of deranged liver function then the standard treatment regimen will be used (100mg/kg/16hr).

Study drug will not be continued.

16.7 Insurance and Indemnity

The Sponsor is responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Principal Investigator and staff. Liability for study medication-induced injury will be according to local requirements. The sponsor will indemnify the Investigator in accordance to national regulations.

The following arrangements are in place to fulfil the sponsors' responsibilities:

- The Protocol has been designed by the Principal Investigator and researchers employed by the sponsor and collaborators. The sponsor has insurance in place which includes no-fault compensation.
- Site participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the Sites concerned. The Sponsor require the site participating in the study to arrange for its own insurance or indemnity in respect of these liabilities.
- Site which are part of the United Kingdom's Nation Health Service will have the benefit of NHS Indemnity.
- The manufacturer supplying IMP has accepted limited liability related to the manufacturing and original packaging of the study drug and to the losses, damages, claims or liabilities incurred by study participants based on known or unknown Adverse Events which arise out of the manufacturing and original packaging of the study drug, but not where there is any modification to the study drug (including without limitation re-packaging and blinding).

17 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

17.1 Study Report

A clinical study report (CSR) will be prepared covering clinical and statistical aspects and summarising all findings of the clinical study. The content has to be treated as strictly confidential. The study report will be sent to the Regulatory Authority and REC according to local requirements.

17.2 Publication

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study after discussion and approval from the Sponsor.

17.3 Publication and Data Rights

Following completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, the Sponsor together with the PI will be responsible for these activities and will decide on how the manuscript is written and edited, the number and order of authors, the journal to which it will be submitted, and other related issues. The Sponsor has final approval authority over all such issues.

Data are the property of the Sponsor and cannot be published without prior authorisation from the Sponsor, but data and publication thereof will not be unduly withheld. It is the intension that any results will be published, both positive, negative or inconclusive.

The published international guidelines for authorship (International Committee of Medical Journal Editors, 1997) will be adhered to i.e. 'All persons designed as authors should qualify for authorship. Each author should have participated sufficiently in the work to take public responsibility for the content.'

Authorship credit will therefore be based only on substantial contributions to 1) conception and design, or analysis and interpretation of data; and to 2) drafting the article or revising it critically for important intellectual content; and on 3) final approval of the version to be published. Conditions 1), 2) and 3) must all be met. Participation solely in acquisition of funding or the collation of data does not justify authorship. General supervision of the research group is not sufficient for authorship. It is intended that information on what each author has contributed will be published.

It is emphasised however, that only those who entirely meet the above mentioned criteria will be listed as authors.

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20 APPENDICES

A: Investigator's Brochure PP100-01

B: Summary of Product Characteristics NAC